

**FTS-CDC-PHPPO**

**October 19, 2005**  
**12:00 p.m. CDT**

Coordinator            Good afternoon and thank you all for holding. At this time, I would like to inform all participants that you will be in listen-only until the question and answer portion of today's conference. Also, today's conference is being recorded. If you have any objections, you may disconnect at this time. I would now like to turn the conference over to Colleen Miller. Thank you, ma'am. You may begin.

C. Miller                Good day. Welcome to our teleconference: Parasitic Telediagnosis, the DPDx System. This is Colleen Miller. I am a consultant with laboratory improvement at the North Carolina State Laboratories Public Health in Raleigh, North Carolina. After the program, each participant needs to register and complete an evaluation form. Documenting your participation helps us to continue to bring high quality training programs in a variety of formats. To do this, please go to the Web site <http://www.phppo.cdc.gov//phtnonline/>. The password is parasites. When

you have completed the registration and evaluation forms, you will be able to print your CEU certificate and you have until November 19<sup>th</sup> to complete this process. These instructions are in your original confirmation letter and the general handout. They were also emailed to each site representative this morning.

If time permits, the end of the program will be open for questions. Remember, you are on a listen-only line. We cannot hear you; you can only hear us. Again, welcome to our program and thank you for joining us. We have over 60 sites from across the United States listening to this teleconference today.

I would like to introduce our two speakers for the program; they are Henry Bishop and Melanie Moser. Melanie Moser is a health communications specialist in CDC's division of Parasitic Diseases and has worked with the DPDx project since 2001. She maintains the DPDx Web site, is involved in the grant process for labs interested in acquiring the DPDx telediagnosis system, and assists in the development of online training for laboratory personnel. Henry Bishop is a microbiologist in CDC's division of Parasitic Diseases and has worked with the DPDx project since its inception in 1998. Along with Henry's duties and the diagnostic

morphology reference lab, he assists in reviewing telediagnosis request, generates the DPDx monthly case studies, and assists in the development of hands-on and online training in parasitology. It is my pleasure today to welcome our speakers Melanie and Henry.

M. Bishop

Good afternoon, everyone. I am Melanie Moser. Some of you know about the concept of telediagnosis or telemedicine and some of you have actually used the DPDx telediagnosis assistance. But for those of you who have not, you may wonder what telediagnosis is and what it can do for you. For those of you that have the equipment, but don't use it or who use it rarely, you may ask why you should use it more often or how you can use it more effectively. I am going to talk about the telediagnosis process, funding for DPDx, and why we feel that telediagnosis is a time and cost effective way of assisting in laboratory identification of parasites. Then, I will turn the presentation over to Henry Bishop who will discuss some of the cases that we have received through DPDx.

Some of your labs may experience higher rates of employee turnover, some of you may not work in parasitology, and some of you may have your job duties and the areas of the lab that you work in rotate periodically. Therefore, there may be people listening today who are not

aware of DPDx. Before getting into the discussion of tediagnosis, I just want to give a brief introduction to the project. If you would all turn to the next slide, which is labeled "Slide 1" in the upper right-hand corner we can begin.

Some of you are probably wondering what kind of name is DPDx? I have noticed that people frequently don't keep the letters in the right order or they might ask what it stands for, so hopefully this explanation will help set it in your mind. Our group is based in CDC's division of parasitic diseases, which is where we get our acronym DPD from. Because part of the focus of our project is on strengthening the diagnosis of parasitic diseases, we merged Dx, an abbreviation for diagnosis into the DPD. That, hopefully, will be a reminder for what DPDx stands for.

Next slide please. Our project was started in 1998 and those of you who worked in parasitology for awhile might recall that in the 1990s, we saw newly emerging parasitic diseases, such as cryptosporidiosis and cyclosporiasis. These two diseases in particular were part of the impetus behind the DPDx project. The project was developed with the purpose of giving parasitology diagnostic assistance to public health laboratories, as I noted before, and also to provide education and training in parasitology.

Those of you who are working in parasitology are aware that there are not a lot of training and continuing education options for those of you who might want or need a refresher.

Probably the most visible aspect of our program is our DPDx Web site and that will give you access to online training and is a place where you can find announcements about our hands-on workshops. You can use the Web site in the same way that you would use a reference text or an atlas to look up information about parasites or to view images. I have included the Web site address on the bottom of slide two for those of you who have not seen our site. But let's go ahead and move on to the actual process of how a telediagnosis request is sent and handled, so please go to the next slide.

There are a few pieces of equipment that are necessary in order to participate in the DPDx telediagnosis assistance. We recommend a digital camera that is mounted to a microscope. On this slide, which is slide three, you will note that the camera has been circled in red. That camera is also connected to a computer that has Internet access. Lab personnel can capture images from the slide they are reviewing, save those images to their computer, and then send them as an e-mail attachment to the DPDx e-mail address. You can ask us for help in making an identification of a

suspected parasite or if you just would like confirmation of an identification you have made.

Next slide please. Along with the images, we ask that you provide other information to us as well. Your name and address are used for our final written report that we send once an identification has been made. We ask that you send your specimen ID code since this is an easy way for us to identify and track cases instead of having to deal with the transmission of more confidential patient information, such as patient name. Any type of information you can give us about the specimen is helpful: when it was taken, what stain was used, the size of the object, and the magnification of the microscopic field captured. Size and magnification are the two things that are most frequently left off of teleradiology requests. As you know, size can be very important when looking at slides or images from an O&P exam. Although you may not get a very complete patient history, any information you can send to us regarding symptoms or travel history is helpful, along with your presumed diagnosis.

Next slide please. The DPDx mail address is for a general mailbox that can be accessed by several people at CDC. I usually manage the mailbox, so when a teleradiology request comes in, I send it to a core group of four

team members. The image on slide five, our core team members are circled in red because I think it is nice to put faces with this sort of anonymous name of DPDx. Our team reads the case history and reviews the images that were sent. Our core team is made up of two microbiologists who work in the parasite morphology diagnostic lab, a Ph.D. in parasitology, and a Ph.D. in molecular techniques who has extensive parasite morphology training. Others in our group provide information technology assistance and assistance in more difficult cases.

Next slide please. We require at least two of our four core members to respond and agree upon an identification before we send a response back to you. This is true of all specimens that are mailed to us as well. We always have two people who review the slides without knowing what the other reviewer's comments and identification are. If your lab doesn't have someone as a full-time parasitologist or if the person working in the parasitology section does not have a lot of experience in that area, teleradiology puts you in touch with experts in our reference lab, and we can help you address cases quickly. For those labs that don't have staffing to allow for a second reader or that have difficult cases, we can serve as a reference lab through teleradiology in the same way that we do when you send us actual specimens.

Our team members are typically available from 7:00 a.m. to 4:00 p.m. Monday through Friday. We are not able to provide assistance on weekends or holidays. Also, if you send a request late in the day, particularly for those of you who are on pacific time, you likely won't get a response until the following morning. We try to answer requests that come in during regular business hours in 30 minutes to at most several hours, depending upon the availability of our staff.

Next slide please. This is slide seven. Along with being able to give assistance to labs that need help in identification of parasites, tediagnosis is also a good screening tool. Even in cases where we can't make a definitive identification from images, we are often able to determine whether we need to see the specimen or whether we will need multiple specimen types. For example, we can rule out non-parasitic organisms based on images and that can save on shipping costs. By reviewing images captured from a slide, we can sometimes determine whether the slide quality is poor or if a case is going to be extremely difficult to make an ID from the slide. In those cases, we can ask for other specimen types. For instance, in a malaria case we could ask for EDTA blood or PCR

along with the slides. We always review slides first before determining whether other types of testing are going to be necessary.

Without seeing images in advance, we may have had the lab send a slide and then later have to request blood, so teleradiology can help speed this process up. Having images also helps us because we can consult with experts outside of parasitology. For instance, sometimes we consult with our pathology group at CDC. In fact, internally we sometimes use our camera to capture images and then we will send out images and a case history to others in our division if we need extra assistance.

Along with screening, the teleradiology and the use of camera can assist in teaching. On images submitted to us, we can point out diagnostic features that the submitting lab may have missed. We can do this via phone while both parties are viewing the same images or by annotating the images with arrows or text to explain diagnostic features. Some of you at the state health departments provide training to county or local health departments and use images captured with your DPDx camera set up in your presentations. Some of you also use the set up for in house training of personnel or to document interesting cases you have received. We would like to encourage the use of the camera in this way.

Next slide please. We have recently reviewed our telediagnosis system and produced this chart to show why telediagnosis makes sense in terms of cost and time effectiveness. If you look at what we call the traditional route for specimen submission, there is time spent by lab personnel in reviewing the slide; then if an identification cannot be made and the specimen needs to be sent to us, there is time and money spent in packaging and shipping the specimen. That specimen might arrive at CDC anywhere from two to three days later, depending upon the mail handler you use.

Once we have received the specimen, we also spend time examining the slides. We estimate that one specimen can cost at least \$95 to send and evaluate, but the minimum turnaround time of 48 hours using what we call the "traditional route". Using DPDx telediagnosis assistance, you still have labor by lab personnel in initially reviewing this slide, but capturing and transmitting the images takes much less time and money, after the initial investment. The review of the images by us takes less time than a traditional microscopy exam. We treat the images and telediagnosis requests as a specimen and we have estimated a cost of \$20 for the process

with a minimum turnaround time of minutes once an image is submitted using DPDx.

Next slide please. If you don't have DPDx equipment or if you need to upgrade your existing equipment, you can request money for the epidemiology and laboratory capacity from Infectious Diseases Cooperative Agreement, which I am just going to refer to as ELC from here on out. ELC's purpose is to improve surveillance for reportable, infectious diseases by providing technical and financial assistance to state health departments. All 50 states have cooperative agreements in place with ELC as well as six large local health departments in major cities in two U.S. territories.

There are two ELC grant cycles each year and states are split up into one of the two cycles. I have included the Web site address for further information about the ELC cooperative agreement is on slide nine. They are supposed to post a listing of states and what cycle you can apply in for your state. I did check their Web site yesterday and they have not yet posted that, but you can always check with the person at your state health department who is in charge of submitting the ELC grant. They should be able to let you know what time of year you can request funds.

Also, please remember that the funding requests for DPDx will come under the food borne disease category of the ELC grant announcements. What I want to emphasize here is that even if you already have funding for DPDx and have the equipment, if it is outdated you can request new funds to either upgrade or replace equipment. This includes cameras or computer equipment. Although, let me clarify that computer equipment only refers to the computer that the camera is attached to, not a computer for your office for instance. Also, if you need to have someone trained in how to use the camera, you can request training money to cover their travel expenses to visit CDC so they can work one-on-one with our DPDx team for a day or two.

If you are at a state public health lab that has large, regional public health departments and if one of those labs sees a lot of parasites, since you are covered by the ELC cooperative agreement, you can request funding for a telediagnosis system on their behalf. This would allow you to set up a telediagnosis system within your state or to let them consult directly with us. Just keep in mind that you will be asked to justify the need for funds in the grant application. California, Florida, Georgia, and Texas all have cameras in two different laboratory locations within their states.

Next slide please. When you request new funding for upgraded equipment, we can provide you with some recommendations about cameras and computer specifications. There are a variety of cameras on the market that can be used for telediagnosis and slide ten shows one in particular that we like. Here at CDC we use the SPOT RT camera that is sold by Diagnostic Instruments. It is a more expensive model than some of the other cameras that we recommend and the camera software is somewhat more complex than software that comes with other cameras, but there are a few advantages. The software comes with a database that allows you to associate up to four categories that you can create and associate with an image. For instance, our categories that we use are parasite, for which we would fill in information such as helminth, protozoa, or arthropod; a second category genus species; also, one for stain used; and a final fourth one for dash number, which is our tracking number. We can then search our database using these categories or you could, if you decided to use a different category structure. Since we have the camera here at CDC and use it regularly, we are able to provide a lot more assistance and tips to states who have this camera.

The next slide, slide eleven, shows a camera that we also have at CDC, which is called the MicroPublisher. It is a more basic camera that is easy to use, although we do prefer the camera software that the SPOT comes with. The MicroPublisher does not allow you to store and search for images in a database. You would have to set up a filing system for images on your computer and then you would have to be careful how you name the images so you can easily identify them or so that you can associate them with your specimen. The MicroPublisher does capture video clips though.

We have also demoed the Olympus DP70 and we were very impressed with the image quality that it is capable of capturing. There are several states out there that have the Olympus magnifier. We personally can't speak to the software or the use of the camera itself, but have not heard complaints about it. We also like to list that as an option.

Next slide please. This slide has a list of computer specifications that we recommend. I don't want to go over all the details, but I want to emphasize the necessity for a large hard drive and at least 512 MB of memory. When you work with a camera and a graphics program, a high amount of memory helps the software run faster. If you are running an older

computer with your camera set up, you might want to ask your IT person to take a look at the computer specifications and request an upgrade through ELC if necessary. Also, if you haven't already, you should consider your image files storage options. You can back up image files to a zip disk or to a CD, but ideally you should save all your work on a network drive so if your computer crashes, you have your work saved elsewhere.

If you send a lot of telediagnosis requests or you use your camera for training local labs or in-house, you want to make sure you don't lose those images. We can speak from personal experience on this because when we first started out with the camera, we were saving images to the hard drive and ended up losing some of our images. Now we always save to the network drive, which our IT people back up every day.

Next slide please. Slide thirteen shows the map that has states that have telediagnosis capacity, but it doesn't show which states actually use it. There are many states that we don't hear from or that rarely send us images, but that still send us specimens through the mail. We would like to encourage them to use telediagnosis more frequently.

I hope I have pointed out how telediagnosis can be useful in speeding up identifications, assisting labs that don't have experienced parasitology staff, and for documenting interesting cases. We are always glad to accept image submissions and case histories for our monthly case studies or to add to our Web site. We are currently revising our image library so if you have some good images that you would like to share, please do so.

Before turning this over to Henry, please go to slide fourteen. I am going to close with just two slides that show usage of the DPDx telediagnosis assistance. Parasitology doesn't get a whole lot of attention in the United States, but it is obvious that there is still a need to maintain proficiency and to have resources such as DPDx to provide assistance. This graph shows the increasing popularity of being able to consult with parasitology experts using digital media and the Internet. Pathologists have been doing this sort of thing for years and really this kind of complication can apply to any area where organism morphology is important and able to be viewed microscopically.

The next slide, slide fifteen, shows that not unexpectedly public health labs are our primary users. But we have seen quite a jump in usage from international laboratories and hospitals in the United States. Being able to

get that quick assistance without sending specimens is bringing the community of parasitologists and microbiologists together in ways that would have been much more difficult before the Internet. I am going to pass the presentation over to Henry who will talk about some of the tediagnosis cases we have received and provide some tips on how to send us a good tediagnosis request.

H. Bishop

Thank you, Melanie. Good afternoon, everybody. If you will go to the next slide please, number sixteen. This is a tediagnosis case that we received from a public health lab. They wrote, "I want confirmation on a malaria specimen I received. The patient is a young adult who traveled to Ghana and stayed there for approximately 17 days." I received slides and a vial of blood. Notice, they are telling us what kind of specimen, basic patient info, although we omitted the ID number for this presentation, and they also included the travel history. They went on to write, "It looks like *Plasmodium falciparum*. The infected red cells are the same size as uninfected. All I have seen are ring forms and there are numerous, multiply infected cells, as well as appliqué forms. Your confirmation would be appreciated." Now, if you will please look at the next two slides, number seventeen and number eighteen, the requester wanted only a confirmation of their identification.

Now, if you will go to slide nineteen, I will go over some of the things that we notice on the pictures they sent. Image A shows a nice ring and the infected red blood cell is indeed the same size as the uninfected cells. Image B shows a multiply infected red blood cell and some appliqué forms. The appliqué forms are noted by the arrows.

Next slide. Here, Images C and D show doubly infected cells and also present are appliqué forms indeed, again shown by the arrows. Also, the size of the infected cells can be compared to the size of the uninfected cells near them so you can see that they are the same size.

Next slide please. You should be on number 21. Images E and D show more ring stage parasites with appliqué forms and Image F, shown again by arrow. These were all classic, morphological presentation of parasites for *Plasmodium falciparum*.

Next slide. Based on the images, we have responses from three DPDx team members and they all agree with an identification of *Plasmodium falciparum*. The requester, in this case, gave us a good case history, including travel, and they also included a presumptive diagnosis. Multiple

images from various fields of the slide were taken, although only images from the thin smear were sent. Some of our more regular requesters do include images from thick smears if they are looking for blood parasites, as well as from thin smears. This requester also made sure that we got a good idea of what uninfected cells look like in comparison to the infected cells.

Next slide please. This telediagnosis came from a university hospital. In fact, the requester was checking to see if we had received an image he sent to us over a month ago, which we had not. As mentioned before by Melanie, we try to respond to all inquiries received during the same business day, during business hours, or at the latest the following day. If you did not get a response from us in 24 hours, you should contact us again. Some labs like to call and let us know that they are sending images and this puts us on the look out for incoming telediagnosis requests and we can be even more responsive.

The patient in this case moved from Africa to the United States. A mass, thought to be a lymph node, was discovered in the groin area. A biopsy of the mass demonstrated a ball of worms. We rarely receive pathology requests, but when we do, we have a few experts in our division who we

can rely on. Like Melanie said, we also have a pathology group here at CDC that we can consult with.

Next slide please. Unlike the first request we looked at, this one included only one image. Unless there are undeniable features that would allow a definitive diagnosis to be made in a single image, it is most always preferred to send multiple images of more than one field or view of area of a slide. Also, the magnification was not given, a critical feature we are to try to determine the size of the organism in this case. Size is always one of the most important features when trying to make a diagnosis based on morphology. Also, there wasn't specific information about where in Africa the patient had moved from. Upon follow up with the requester, they did not have a good patient history either.

Next slide please. Here on slide 25, we have the same picture that we have annotated. Thanks to our nematode expert and one of our parasitologists, both who are members of our senior staff, we were able to make an identification of the worm in this image as being an adult female *Onchocerca volvulus* worm. The diagnostic features seen include a presence of a thin cuticle, shown by the red arrows, and paired uterine tubes, shown by the black arrow. These features, along with travel to

Africa, location of the worms, and general appearance of the morphological features allowed them to make this call.

Next slide please. This is another of our recent requests that came in from an international location. These images were sent in a PowerPoint presentation and also a Word document, so the image file sizes were not very large. In this case, the requester is not sure of what to make of the structures and wrote, "I am sending some pictures of a 'parasite' they found in feces in a hospital that they are strange looking formations." They are asking, "Can it be *Strongyloides stercoralis*?"

Next slide please. Depending upon what organism is depicted, it may be more difficult to examine images due to this way as opposed to getting them sent as image files because, as image files, we have more flexibility in how we view the images and even we can enhance the images a bit if needed. This is rarely possible with images imbedded in a PowerPoint presentation or in a Word document, but it is an option you can consider when sending a telediagnosis. It allows you to easily annotate the images if you wish and you can also type the case history into the presentation.

Next slide please. Like the previous telediagnosis request of Onchocerca, no magnification was given and no patient history was included. Most importantly, no size and measurements of the object were given in the text. Our team members were still able to say, “No parasites found; probable plant material based on these images.” No internal structures, such as organs are evident. It is the most obvious feature shown or rather not shown in the images.

Next slide please. This case was sent to us from a private health center. The requester suspected Plasmodium species and gave us good information about the specimen and a travel history, but the images were too small to make out any details. We did not adjust the image size for this presentation. They are at the actual size that we received them. In fact, some of the pictures look like they are the same, so either an error was made when attaching them or our request for multiple images was confused with sending us more than one copy of the same image. In this case, we were unable to say anything about these images and we responded with a request for larger images. The black border around some of the edges of the images make it look as if these are 35mm slides that have been scanned.

There are a few ways to get around having a digital camera mounted to a scope. We have had people send us images from hand-held digital cameras that were held up to the microscope ocular, although this takes some practice and skill. We have also had one requester take pictures with a 35mm camera mounted to his microscope and then take the film to be developed at a one-hour photo shop. They scanned the printed photos and sent us the image files via e-mail. This method can work especially if the person taking the images has a steady hand and a decent camera. But it is important to remember that image quality is instrumental for us to make an identification.

Next slide please. This is a case of Babesiosis that we received through one of our epidemiologist who was working with the patient's attending physician. You can feel free to look through slides 30 through 35 while I discuss the case history. The case scenario involves an elderly patient who lives in a state that has Babesia endemic areas. Several months before the images were submitted to us, the patient had been diagnosed with Babesiosis by blood smear, was treated, and had improved.

Along with this information, we were told that the patient had no recent history of a tick bite or exposure, but that he was experiencing recurrent

symptoms. The spleen had been removed several years ago and he had a blood transfusion during his recent hospital admission. His travel history included numerous trips between his home state and southern states by car over a time period of several months. His most recent specimens have been sent to a private lab and they stated the patient was IGG, IGM positive for Babesia microti, but his PCR results were negative. We were asked to determine if this was a case of B.microti or if this was another species of Babesia.

Now if you will please advance to slide 36. We agree that this was indeed a case of Babesiosis. However, because it is next to impossible to speciate Babesia based on microscopy, serum and EDTA blood was requested and sent to our reference labs here at CDC. Our immunodiagnostic reference lab performed their test for B.microti and the result was negative. They then performed a serologic test for Babesia species and got a positive result. Our molecular lab also performed PCR on the blood and got a positive result for Babesia sub type MO-1. It is likely that in his travels between his home state and the south that the patient did have tick exposure, but was unaware of it. We would like to thank Dr. Chris Pfeifer, the attending physician, and David Fortinac, the Laboratory

Medical Technologist, who sent us this telediagnosis for allowing us to use this case today.

Next slide please. You should be on slide 37. This telediagnosis request was sent by a public health lab and was preceded by the actual specimen. The requester had sent serum, stool, and BAL fluid to our morphology diagnostic lab, but then followed up with images they had previously taken. Patient information was included with the paperwork that arrived with the actual specimens, but was not in the e-mail with the images. Image A was captured from a cytology pap specimen.

Next slide please. Image B was captured from a gram stain of a sputum sample. Image C shows the short buckle capsule, which has been annotated with a circle. Image D shows filaria formed larva in the circles. The requester named the image files according to either specimen type, for example cytologypap.jpg or feature, shortbucklecapsule.jpg. This is a good way of communicating the information. Some of our requesters use their specimen ID code as the image name followed by a number or alphabetical listing. This is also a useful way of naming image files because we need the specimen ID code for our record and it is a good way for the submitting lab to associate the case and images from the case.

Next slide please. We agreed that the larvae looked like *Strongyloides stercoralis*, but we wanted to examine the specimens that we had received and we also wanted to perform serology. This telediagnosis included some good information, but we did not get staining and sample information for Images C and D. The requester also left out an important piece of information, the size of the objects or larvae.

Next slide please. Upon receiving the BAL specimen, we made a smear and saw a few larvae that had morphological features consistent with *Strongyloides stercoralis*. Here the arrow points at the genital primordium. In cases where we received the actual specimens, we can always capture images from them, annotate the images to point out diagnostic features we observe, and send the images back to you. In this case, our serology lab also got a positive result for *Strongyloides*.

Next slide please. This case was a telediagnosis from a public health lab. It highlights one of the potential pitfalls that could occur by relying solely on digital images to make a diagnosis and it emphasizes the need to pay attention to all patient information that you receive, which this requester did not.

M. Moser                    He did.

H. Bishop                    Did. I am sorry. This submission had good case history and multiple images. The submitting lab suspected P.vivax, but stated that the hospital that referred the specimen to them had diagnosed the patient with P.falciparum. Images A and B show trophozoites that are little amoeboid and enlarged red blood cells.

Next slide please. Slide 43 shows Images C, D, E and F. As you can all see, large gametocytes and noticeably enlarged red blood cells.

Next slide please. Image G shows another gametocyte. Images H and J show more trophozoites, some in not so enlarged red blood cells. Image I shows a schizont with about nine or so meronts or merozoites.

Next slide please. Those were the images submitted by the public health lab. Since the hospital suspected falciparum, but the images only indicate a probable vivax or ovale, we asked for smears and blood to be sent to us. The following week on the same day that the specimens arrived at CDC, we also received a follow up teleradiology from the reference lab.

Next slide please. The lab had gone back and reviewed the thick smears and found *P.falciparum* gametocytes. Recall that I mentioned that you should always pay attention to all information that is given to you by the submitting lab and this requester did. They remembered that the hospital stated that they saw *P.falciparum*, so the lab had gone back and reviewed the thick smears and found *Plasmodium falciparum* gametocytes. These images show the typical banana-shaped gametocytes for *Plasmodium falciparum* in slides 46 and 47.

Next slide please. You should be on slide 48. We could confirm the identification of *Plasmodium faciparum* from the images and also from the smears we received, but we were still unable to speciate between *Plasmodium vivax* or *ovale* from the smears or the images, so the blood was sent for molecular testing. The final identification was a mixed infection of *Plasmodium falciparum* and *Plasmodium ovale*. This case shows a good collaboration between the public health lab and CDC. By paying attention to all the case information, the submitting lab continued to examine specimens and sent us images of what they found even though they recognized that PCR testing was necessary and they had to send the specimen.

Next slide please. Along with routine requests, telediagnosis can be useful in outbreak situations. Slide 49 shows that we received images in a PowerPoint presentation from a public health lab.

Next slide please. The specimen identifiers and a presumed diagnosis were given in the text of the e-mail, but size and staining were not mentioned. Although it seemed apparent that for each specimen there was one UV fluorescent image and one image taken from an acid-fast smear, it is still helpful to include this information to confirm our suspicions.

Next slide please. You should be on slide 51. At this time, I would like to discuss the training applications that your camera can be used for. Slide 51 has a case scenario involving a 70-year old man who was seen by an ophthalmologist for eye pain. Upon examination, the doctor found and removed a subconjunctival worm that was enclosed in a cyst. The man had no recent travel outside the United States, but had traveled to Central America over five years ago, though, and reported that he did have pets at home. The image shows the cyst and the worm it contained. The worm was approximately 92mm in length. This image was taken here using a handheld camera held up to an ocular of a dissecting microscope. This

was mentioned earlier in the presentation as an alternative to a dedicated, mounted digital camera on your microscope.

Next slide please. We transferred the worm to a depression slide and Images B and C were captured with the dedicated camera mounted on a compound microscope. The magnification is given in the caption of the images.

Next slide please. The morphological features we observed enabled us to make an identification that this was a male filaria worm in the genus *Dirofilaria*, most likely *Dirofilaria tenuis* based on absence of travel history and given the geographical location of the patient's residence. The presentation of the worm and the cyst is not typical, but the morphology of the worm, including the size of it was consistent with the features of *Dirofilaria*.

Next slide please. Here on slide 54 what we did is we captured images and then annotated them to highlight key points that we observed. Here we point out some of the key features. The green arrows point to the caudal papillae of the tail shown in Image B, the red arrow points to a

spicule. We then explain that the presence of papillae and spicule indicated that this was a male worm.

Next slide please. In Image A, the ridging and striations of the cuticle appear beaded sort of a in a cornrow effect, shown by the blue arrow, indicating that the worm belonged to the genus *Dirofilaria*. Notice that we also included the magnification used. This is the kind of training application that works when the specimen is forwarded on. Now, in the next case, we were able to offer guidance without have the specimen sent to us for observation.

Next slide please. In slide 56, there is a case involving a 39-year old female who had a descending colon biopsy to determine the cause of symptoms that included chronic abdominal pain and discomfort. No pertinent travel history was known, worm-like objects were detects during the biopsy, and some were recovered. The specimens were sent to a state health department laboratory for identification. Lab personnel at the state took digital images, figures A through C, and sent them to DPDx at CDC for assistance in making that identification via telediagnosis. This case was also used as one of our monthly case studies on DPDx and that is why

you see on your slide the questions, “What is your diagnosis based on what criteria?”

Next slide please. We suspected that this was a case of Enterobiasis caused by *Enterobius vermicularis*, otherwise know as pinworm. Diagnostic features we could see were the presence of cephalic inflations on the anterior end, indicated by the arrows we added to the image that they had submitted.

Next slide please. Here on slide 58, other features were the absence of spicules in the images of the tail, suggesting that this was a female worm. The tail of a female pinworm is long, tapered, and slender. Also, the presence of eggs in the exposed uterus confirmed that this was a female pinworm. However, diagnostic features of the eggs could not be determined by the magnification at which the images were taken.

Next slide please. Therefore, the DPDx team asked laboratory personnel at the South Carolina Bureau of Laboratories to try to tease out some of the eggs, photograph them, and submit an image to verify the initial diagnosis we had given them of *Enterobius*. The personnel promptly complied and the following image submitted of the egg below shows a

typical pinworm egg, based on size 50x60x20 to 32 micrometers and shape, oval with one side flattened, adding the final confirmatory feature to this case.

Next slide please. In summary, we leave you with a few tips. When using lower magnification objectives, for example, 10X through 40X, you can increase the contrast of the image by adjusting the condensers aperture setting according to the objective you are using. You should always do a white balance prior to an image capturing session or when switching between differently stained slides. This is part of your camera's software. This ensures accurate color reproduction. You should always make a copy of your image file before using image-editing software to alter the image, be it even just decreasing in size. Although not written on this slide, we recommend that you save your original image file as a tif. You can also reduce file size by saving images as jpegs. Of course, please contact us for additional instructions and tips on image capturing.

This concludes our presentation on DPDx telediagnosis assistance and we would like to thank you for tuning in and listening to us. We also would like to thank the NLTN for inviting us to speak on this topic. At this time,

I suppose it is time to turn it back over to the moderator and open up the lines for any questions you may have. Thank you.

C. Miller Thank you, Melanie and Henry. We would like now to take your questions. The operator will take over from there and will instruct you how to dial to do questions.

Coordinator Yes. That is correct. One moment for the first question.

C. Miller Do we have any questions? I think I was so enthralled by the pictures, I got off a little bit in what I was supposed to say. But if you do have any questions, please feel free to ask.

Coordinator We do have one question from Mona Mandour. Please go ahead.

M. Mandour Yes. I was wondering what is a white balance? We take pictures and we have a camera and all that, but I am not quite sure what that means, always do a white balance prior to your image.

H. Bishop What kind of camera do you have?

M. Mandour A SPOT.

H. Bishop A SPOT?

M. Mandour Yes.

H. Bishop There is a little triangle with the three primary colors on your toolbar. Basically, what it does is it calculates the amount of light coming in and it puts everything in its proper perspective. It tells you that you should move to a fairly clear space on the slide before you do your white balance and it is going to come up with certain values for red, green, and blue. Then it will ask you to save that to your image set up. What I do is I just have a plain set up called "working" and I just save it to that. If you don't do a white balance, you will notice, especially if you were to take images from an acid fast slide, that the reds do not look as red as they should. Have you taken any images where the colors just didn't look right?

M. Mandour Yes.

H. Bishop Next time, like I said, move to a fairly clear space on the slide and go to your toolbar. It is a little triangle and it has red, green, and blue. Just

click that and it will say if you move to a clear space on the slide and say “yes” and go ahead and click and begin. It will take a few seconds, then some values will pop up, and it will ask you to save those values. It will probably default to your factory default and that will be fine. Just save it wherever you want to.

It will be easier if we could set up a teleconference at some later date and time and I can walk you through this step-by-step. It really helps in keeping with color reproduction, but it is easier if I go on my camera and microscope and we do it step-by-step. That way, you know exactly what I am talking about as far as where to save it, when to move the slide, etc. In a nutshell, it just corrects the color.

M. Mandour

Thanks a lot.

H. Bishop

You're welcome.

Coordinator

Thank you. Our next question comes from Jeffrey Greenberg.

J. Greenberg

Hello. My question is what are you suggesting that each state should have equipment and then the lab should send it to us?

M. Moser                    In fact, most of the states in the U.S. already do have the equipment.

                                  What we suggest is that if they have cases for suspecting parasites that they want help identifying with, they can send images to us. Also, within states, as I said if you have a large regional lab or a lab that might see a lot of parasitology, you have a lab that is doing, for instance, maybe refugee screening or something like that, as long as they are a public health institution, if you are at the main state health department, you can request funding for a teleradiology system for a different lab in your state. But you would have to justify the need for it.

J. Greenberg                Sure. Okay. Thank you.

M. Moser                    They could use you as the reference lab or they could send images to us.

J. Greenberg                Thank you.

Coordinator                Thank you. Our next question comes from Diane Schultz.

D. Schultz                    Hello. A question: do you charge to review the pictures we send to you?

M. Moser                    We do not charge. It is free of charge.

D. Schultz                The mailbox that we send it to. Where do we send it to?

M. Moser                    Actually, the e-mail address is [dpx@cdc.gov](mailto:dpx@cdc.gov) and I believe it is the very last slide in your presentation. There is a little blurb that says for more information and it has our e-mail address and our Web site.

D. Schultz                Thank you.

M. Moser                    Sure.

Coordinator                Thank you. Our next question comes from Joseph Guzman.

J. Guzman                 Hello. On slide 49 for the telediagnosis of outbreaks, what was the final identification or were you able to identify based on those images?

H. Bishop                 Slide 49?

J. Guzman                 Yes.

H. Bishop            It was Cyclospora, sorry.

J. Guzman            Thank you.

H. Bishop            You're welcome.

Coordinator           Thank you. I am showing no further questions at this time.

C. Miller            If there are no further questions or if you didn't have your question answered, please e-mail this Web address [neoffice@nltn.org](mailto:neoffice@nltn.org) and the speakers will be glad to answer your questions by the e-mail address.

I do have a reminder. I would like to just remind you that all participants that are listening to our program today to please register and complete an evaluation form by November 19<sup>th</sup>. The directions for this are on your confirmation letter and general handout. They were also e-mailed to each site representative this morning. Documenting your participation helps us to continue to bring high quality training programs in a variety of formats. When you have completed the registration and evaluation form, you will be able to print your CEU certificate. That concludes our program today.

I would like to announce our next teleconference will be on November 16<sup>th</sup>. The topic is the Food Emergency Response Network, FERN. The co-sponsors of today's program would like to thank our speakers, Melanie Moser and Henry Bishop. Thank you for joining us. I hope that all of you will consider joining us for future programs and that you will make the National Laboratory Training Network your choice for laboratory training. From the North Carolina State Laboratory of Public Health in Raleigh, North Carolina this is Colleen Miller. Good day.

Coordinator

Thank you. That concludes today's conference. You may disconnect at this time.